

Ontogeny, life history and sex ratio evolution in *Ensliniella kostylevi* (Acari: Winterschmidtidae)

J. S. H. KLOMPEN¹, F. S. LUKOSCHUS² AND B. M. O'CONNOR¹

¹Museum of Zoology and Department of Biology, The University of Michigan, Ann Arbor, Michigan 48109–1079, USA ²Department of Aquatic Ecology, Catholic University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands

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(With 1 plate and 5 figures in the text)

The nest-inhabiting ontogenetic stages of the mite *Ensliniella kostylevi* Zakhvatkin, 1941, an associate of the eumenine vespid *Allodynerus rossii* (Lepeletier, 1841), are described, with a redescription of the phoretic deutonymph. Observations on the life history of *E. kostylevi* show the presence of male dimorphism and a very high degree of correlation in developmental timing of the mites and their hosts. This life history is compared to that of related species, with an emphasis on the variously biased sex ratios observed among the oviparously produced adults. The extent of this bias is hypothesized to be directly related to the number of founders in each wasp cell, a number which, in turn, is largely determined by morphological and behavioural characteristics of the hosts.

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Introduction

The close association of mites in the genus *Ensliniella* Vitzthum, 1925 (Winterschmidtidae) with eumenine wasps has been recognized by numerous authors (Enslin, 1922; Vitzthum, 1925; Cooreman, 1942; Benno, 1945; Crèvecoeur, 1945). The developmental timing of these mites, most of whose life stages live in the wasp cell, is almost perfectly correlated with that of the immature wasps (Vitzthum, 1925; Cooreman, 1942). Unfortunately, none of these studies, nor subsequent descriptive studies by Zakhvatkin (1941) and Baker & Cunliffe (1960), have provided adequate descriptions of the nest-inhabiting life stages, concentrating instead on descriptions of the phoretic deutonymph. However, in order to place these studies on life history of these mites in an evolutionary context, more on the systematic background needs to be known, including descriptions of all life stages. The discovery of a number of nests of *Allodynerus rossii* (Lepeletier,

1841) infested with the mite *Ensliniella kostylevi* Zakhvatkin, 1941, allowed both the first descriptive study of all life stages and observations on the life history. Given these data on systematics and life history, it is now possible to present a comparative study of life histories in the known eumenine associated Winterschmidtidae.

The genus *Ensliniella* was proposed by Vitzthum (1925) to accommodate a new species of mite, *Ensliniella parasitica* Vitzthum, 1925, found in the nest of the solitary wasp *Allodynerus delphinalis* (Giraud, 1866). Zakhvatkin (1941) redescribed this deutonymph and described the deutonymph of a second species, *E. kostylevi*, phoretic on *A. rossii* (Lepeletier, 1841), collected in the USSR (Crimea). Interestingly, Vitzthum (1925) had already mentioned mites found on this host species, but he considered them conspecific with *E. parasitica*. *Ensliniella kostylevi* has since been found on *A. rossii* collected in Belgium (Cooreman, 1954), The Netherlands, France, Austria, Bulgaria, Greece and Turkey (all during the present study). These collections indicate that the range of this mite coincides with that of its host: Europe, Morocco, Turkey, south-western USSR and Iran (van der Vecht & Fischer, 1972; Giordani Soika, pers. comm.).

Observations on the life history of the eumenine-associated Winterschmidtidae range from the early work of Vitzthum (1925) and Cooreman (1942) on *E. parasitica* to the more recent work of Krombein (1961, 1967) and Mostafa (1970) on several species of *Vespacarus* Baker & Cunliffe, 1960 and on *Monobiacarus quadridens* Baker & Cunliffe, 1960. However, all of these observations are restricted to short notes on life history. The only species for which detailed studies have been carried out is *Kennethiella trisetosa* Cooreman, 1942, associated with *Ancistrocerus antilope* (Panzer). Both the period on the adult wasp (Cooper, 1955) and the period in the nest (Krombein, 1967; Cowan, 1984) are well documented, showing the existence of male dimorphism and complex interactions between the mites and their hosts. Our observations on the life history of *E. kostylevi* will focus on a comparison with *K. trisetosa*, in particular in relation to the evolution of the sex ratio of oviparously produced adults.

Materials and methods

Through the aid of the Consulentenschap van de Dienst voor Bijenteelt (Institute for Apiculture) in Hilvarenbeek, The Netherlands, one of us (FSL) obtained a number of nests of *Allodynerus rossii* (Lepeletier, 1841) (Hymenoptera: Vespidae), from reed stems used in a roof. Many of these nests were infested with the mite *Ensliniella kostylevi* Zakhvatkin, 1941. Both wasps and mites were reared to obtain all ontogenetic stages of the mites. Half of the stems were kept at 30 °C, the rest at 20 °C. The nests were checked daily and several collections of mites were made during the study period. Our observations on *E. kostylevi* span the period in the nest of the wasp host from late winter until the emergence of the adult wasps in spring. Wasps and mites were killed and preserved for further study a few days after eclosion of the wasps. The wasps are deposited in the collection of the Rijksmuseum van Natuurlijke Historie, Leiden. Since the initial goal of this study was limited to obtaining all ontogenetic stages of the mite, no quantitative data were kept.

The nomenclature used in the descriptive part of this study follows Fain (1963) for the body chaetotaxy, and Grandjean (1939) for the leg chaetotaxy. All measurements are in micrometres (μm) and summarized in tabular form (Table I).

Morphology of *Ensliniella kostylevi*

Description of ontogenetic stages

Ensliniella kostylevi Zakhvatkin, 1941: 367–368 (Plate I; Figs 1–5)

FEMALE (Figs 1, 5a-c). Body oval-shaped with weak sclerotizations. Average length of 16

specimens 591, standard deviation (S.D.) 51; average width 407, S.D. 46. *Dorsum* (Fig. 1a). Propodosomal shield weakly sclerotized, laterally indented; length subequal to width. Sejugal furrow poorly developed. Dorsal setae in live specimens almost perpendicular to the body. Setae *scx* small and smooth; most other dorsal setae long and whiplike (setae *vi* and *l*₄ relatively short); setae *l*₃, *l*₄ and *l*₅ inserted latero-ventrally. Duct of the bursa copulatrix (BC) short, ending externally in a small, rounded papilla (Fig. 4). *Venter* (Fig. 1b). Coxal apodemes small; apodemes of coxae I fused to form a short sternum; apodemes of coxae II, III and IV free; apodemes of coxae IV curved inwards. Opisthosomal glands large, opening near setae *l*₃. Coxal setae *cx I*, *cx III* and *cx IV* short and filiform; setae *sh* long and whiplike. Genital area between coxae III; genital opening shaped like an inverted 'V' bordered by genital valves; two pairs of genital papillae, two-segmented with blunt ends. Setae *ga* and *g* short and filiform. Anus subterminal, covered by two valves. Two pairs of anal setae present (*a*₂, *a*₃), elongate and filiform, positioned, respectively, anterior and lateral to the anus.

Chelicerae and gnathosoma (Fig. 1b, c). Chelicerae chelate-dentate; both fixed and movable digits with five teeth; cheliceral spur absent; one pair of cheliceral setae on the paraxial surface. Palps one-segmented with a membranous portion midway between base and apex. Two dorso-lateral setae, one ventral seta, one eupathidial seta, ventro-apical on top of a small tubercle, and a very small dorso-apical solenidion are present on each palp. Lateral lobes of the rutellum with one distinct and one very small tooth. Subcapitulum with a single pair of ventral setae.

Legs (Fig. 5a-c). All legs of similar length, with five free segments. Chaetotaxy: trochanter I, II and III with setae *pR* and *sR* elongate and filiform; femora I-II with a very long seta *vF*, seta *wF* IV shorter; genual setae *cG* I-II, *mG* I-II and *nG* III elongate and filiform; setae *gT* and *hT* on tibiae I-II filiform, distinctly shorter than seta *kT* III-IV. Tarsi I-II with nine setae: setae *p* and *q* stout spines; seta *s* short and spinose; other tarsal setae (*d*, *f*, *e*, *wa*, *ra*, *la*) elongate and filiform. Tarsus III with eight setae: setae *p*, *q* and *s* as on tarsi I-II; setae *d*, *w* and *r* long and filiform, setae *f* and *e* much shorter. Tarsus IV as tarsus III, but setae *f* and *e* absent. Solenidia: solenidion sigma-1 on genu I long and tapering, sigma-2 elongate but smaller; sigma-1 on genu II distinctly shorter than on genu I. Solenidion phi present on all tibiae, long and tapering. Tarsus I: solenidion omega-1 elongate with a rounded tip and a short famulus epsilon (length 2-3); omega-2 short, positioned posterior basal to omega-1; omega-3 long and tapering, near the apex of the tarsus. Tarsus II with solenidion omega-1, but without a famulus. All pretarsi with a well developed ambulacrum; empodial claw protruding from a bell-shaped ambulacral disc; condylophores fused basally, limited to the ambulacral disc.

MALE (Large type; Figs 2a, 3a, c). Average length for 10 specimens 444, S.D. 21; average width 296, S.D. 14. The male resembles the female in all but a few characters. Body size smaller; average setal length smaller (Table I); apodemes of coxae III and IV longer than in the female, with the anterior ends of apodemes IV close to each other. Genital area between coxae IV, showing heavy sclerotizations; aedeagus thick, average length for four specimens 28 (S.D. 1); genital papillae as in female. Setae *ga* and *g* absent, but vestigial alveoli present anterior to the aedeagus (Fig. 3c). Gnathosoma and legs as in female.

MALE (Small type; Figs 2b, 3b, d, 5d). Average length for three specimens 289, S.D. 5; average width 174, S.D. 5. This type of male differs from the large type male in significantly smaller overall size (*P*-value < 0.005 (*d.f.*: 11) in Student's *t*-test), relatively shorter dorsal setae (ratio of the median dorsal setae/body length 0.25-0.30, in large type males 0.35-0.40; difference significant: *P*-value 0.003 (*d.f.*: (1, 4)) in a three-way analysis of variance using ratios for setae *sci*, *d*₁ and *d*₂), the presence of a third pair of anal setae (*a*₁) anterior to setae *a*₂ and *a*₃ (Fig. 3b), the presence on

TABLE I
Measurements on the life stages of *Ensiiniella kostylevi* Zakhvatkin, 1941

	Female			Male Large type			Male Small type			Deutonymph			Protonymph Large type			Protonymph Small type			Larva Large type			Larva Small type
	N	Av.	S.D.	N	Av.	S.D.	N	Av.	S.D.	N	Av.	S.D.	N	Av.	S.D.	N	Av.	S.D.	N	Av.	S.D.	ms
Total length	16	591	51	10	444	21	3	289	5	40	266	15	11	299	13	4	257	12	11	192	14	-
Body width	16	407	46	10	296	14	3	174	5	40	179	13	10	206	11	3	156	3	11	126	12	-
Propodosomal shield length	8	101	6	5	88	5	2	59	5		a			a			a			a		a
Propodosomal shield width	8	108	5	6	104	6	3	61	8		a			a			a			a		a
Seta <i>vi</i>	4	102	5	4	79	8	1	40	-	8	7	1	5	23	5	3	50	3	5	23	2	28
Seta <i>sci</i>	4	178	19	5	158	19	3	78	1	10	6	1	5	65	9	3	109	3	5	53	4	-
Seta <i>sce</i>	5	185	18	5	158	14	3	85	3	10	10	2	5	78	6	3	107	9	5	57	7	-
Seta <i>scx</i>	5	23	5	5	17	2	2	11	2	9	11	1	5	10	1	3	15	3	5	10	1	-
Seta <i>d 1</i>	5	178	20	5	174	15	3	77	12	9	6	1	5	71	7	3	114	6	5	43	10	37
Seta <i>d 2</i>	5	188	18	5	164	10	3	76	18	8	6	1	5	74	8	3	103	1	5	44	6	37
Seta <i>d 3</i>	5	185	23	4	161	18	2	75	4	9	7	1	5	69	11	3	97	5	5	39	1	30
Seta <i>d 4</i>	5	184	16	2	146	13	1	54	-	7	7	1	5	63	6	3	96	13	5	30	4	-
Seta <i>d 5</i>	5	186	19	4	146	19	3	78	7	8	8	1	5	63	6	3	105	15		a		a
Seta <i>l 1</i>	5	201	30	5	174	13	3	82	4	9	6	1	5	66	11	3	110	10	5	44	8	36
Seta <i>l 2</i>	5	205	15	5	164	14	3	90	12	6	6	1	5	74	6	3	101	1	5	33	7	27
Seta <i>l 3</i>	5	180	22	4	163	12	3	79	13	9	6	1	5	60	5	3	106	8	5	23	2	-
Seta <i>l 4</i>	5	143	19	4	128	29	2	42	8	9	4	1	5	14	1	3	37	6	5	43	5	-
Seta <i>l 5</i>	5	207	20	4	182	8	2	81	13	10	28	3	5	58	5	3	87	3		a		a
Seta <i>h</i>	4	203	21	5	174	23	3	95	12	10	46	5	5	73	11	3	112	2	5	43	3	35
Seta <i>sh</i>	5	102	16	5	103	11	3	39	5	8	4	1	5	24	1	3	48	3	5	15	3	11
Seta <i>a 1</i>		a			a		2	7	1		a			a			a			a		a
Seta <i>a 2</i>	5	73	4	3	46	9	2	20	1		a		5	17	1	3	20	1		a		a
Seta <i>a 3</i>	5	62	4	4	45	3	2	16	2		a		5	12	2	3	22	5		a		a
Seta <i>cx I</i>	5	52	6	5	35	3	3	18	1		a		5	17	1	2	18	1	5	15	1	-
Seta <i>cx III</i>	4	51	4	5	34	3	3	17	1		a		5	15	1	2	20	4	5	12	1	-
Seta <i>cx IV</i>	4	51	4	4	32	4	3	17	2		v			a			a			a		a
Seta <i>ga</i>	5	50	3		a			v/a		7	33	7		a			a			a		a
Seta <i>g</i>	4	44	7		a			v/a		8	4	1	5	13	1	2	17	2		a		a
Omega-1 I	5	28	1	5	26	1	3	15	2	10	21	1	5	13	1	3	16	4	3	9	1	7
Omega-2 I	5	9	1	5	7	1	3	3	1		v/a		4	3	1	3	4	1		a		a
Omega-3 I	5	35	1	5	34	1	3	20	3	10	41	2		a			a			a		a
Phi I	5	96	5	5	82	7	3	55	1	9	59	4	5	45	3	3	51	5	5	42	2	31
Sigma-1 I	5	66	5	5	51	8	3	33	2	10	7	1	5	23	2	3	26	2	4	17	1	13
Sigma-2 I	5	11	1	5	9	1	3	5	1		v		4	3	1	3	4	1	5	4	1	3
Sigma-1 II	5	29	1	4	25	1	3	12	1	6	15	1	4	10	1	3	13	3	3	5	1	6

Av. = Average; S.D. = Standard Deviation; ms = measured specimen
v = vestigial alveolus; a = absent; - = no measurements due to the poor condition of the specimen

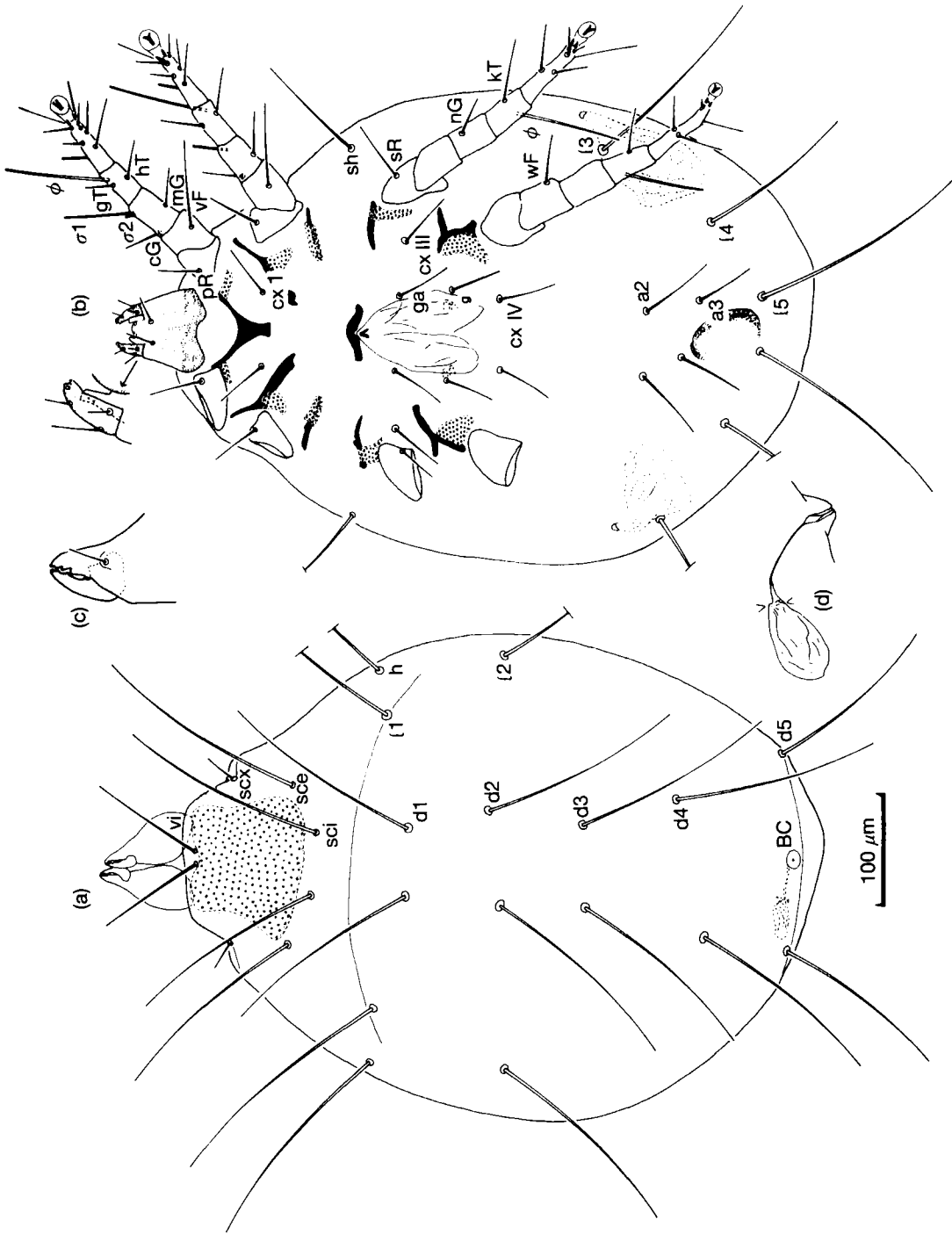


FIG. 1. (a-d) *Ensliniella kostylevi* Zakhvatkin, 1941. Female. (a) Dorsal view; (b) ventral view; (c) chelicera; (d) bursa copulatrix.

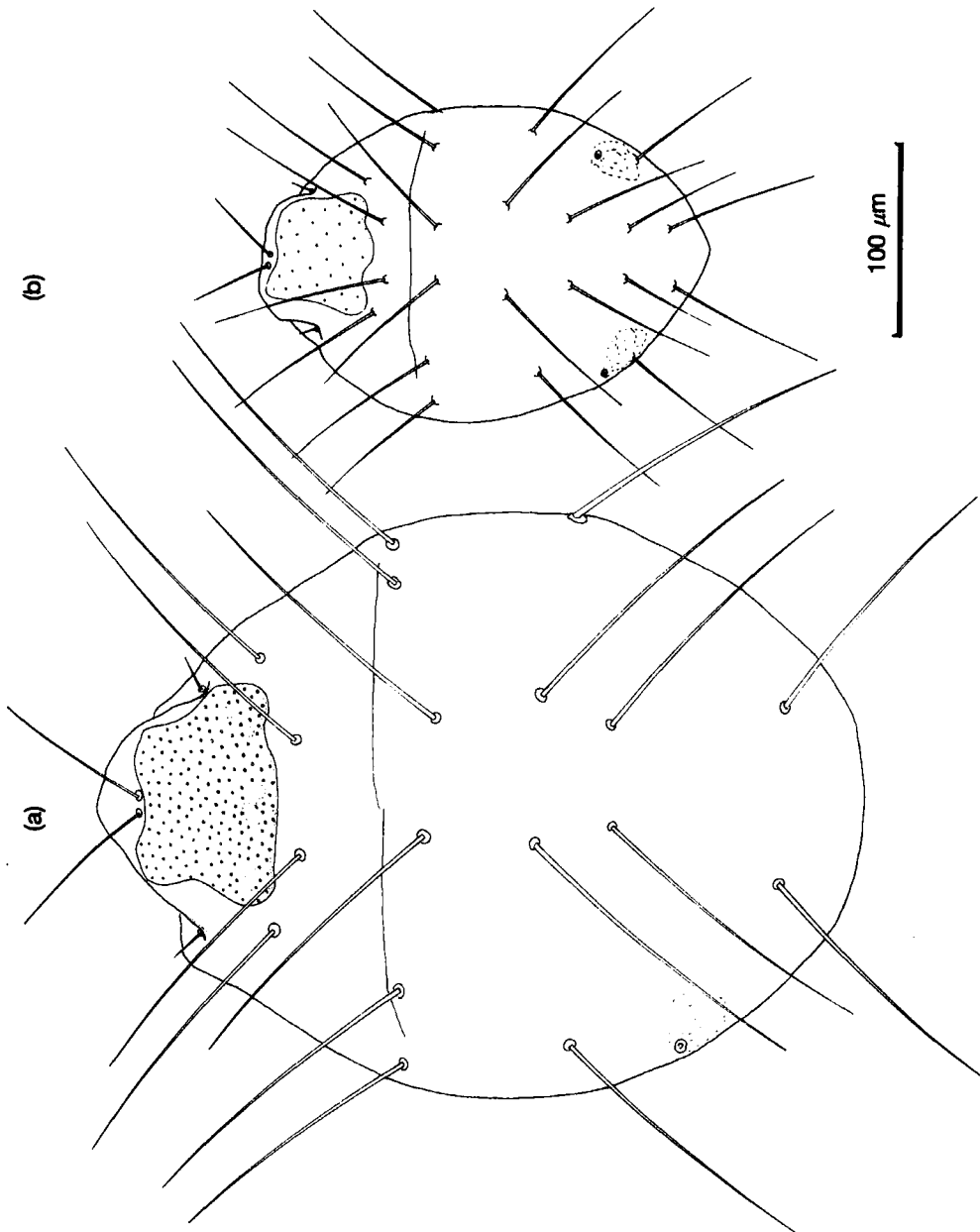


FIG. 2. (a, b) *Ernstiniella kostylevi* Zakhvatkin, 1941. Male. (a) Large type male, dorsal view; (b) small type male, dorsal view.

tarsus II of a second solenidion close to solenidion omega-1 (Fig. 5d), the presence in some specimens of setae *ga* and *g* (Fig. 3d) and the weak sclerotization of the propodosomal shield and genital area. Structure and position of the genital area as in the large type male; aedeagus complete in only one specimen, length 16.

TRITONYMPH (Large type). This stage has not been observed, since the rearing period did not include the phase in which the mites enter a new cell. Presumably, this is the period in which this stage would be present.

TRITONYMPH (Small type). This stage has been observed but was not collected.

DEUTONYMPH (Figs 4a, b, 5e-g; Plate Ia-e). Average length for 40 specimens 266, S.D. 15; average width 179, S.D. 13.

Dorsum (Fig. 4a). Sejugal furrow well developed. Dorsum almost completely covered by a propodosomal and an opisthosomal shield. Both shields profusely micropunctate with numerous shallow striae. Propodosomal shield with reticulate striations (Plate Ia) and almost triangular in shape. Opisthosomal shield with transverse striations in the anterior region, changing to longitudinal posterior to the *d*₂ setae; maximum width subequal to length; the posterior edge of this shield is strongly sclerotized with a median apodeme pointing anteriorly, not reaching the level of setae *d*₄. Large eyes are present on the anterior edge of the body with heavily pigmented retinas. Propodosomal setae short and filiform; setae *vi* and *sex* not on the propodosomal shield; setae *scx* short and smooth. Opisthosomal setae *h* and *l*₅ long and whiplike, other opisthosomal setae short. Cupules *ia*, *im*, *ip* and *ih* present.

Venter (Fig. 4b). Palposoma absent; solenidion alpha twice the length of the palposomal seta (Plate Ib). Coxal apodemes well developed; apodemes of coxae I fused to a long sternum; apodemes of coxae II free, the posterior apodemes II almost reaching coxal apodemes III; anterior apodemes of coxae III and IV fused to a median ventral apodeme which extends posteriorly past setae *cx IV*; posterior apodemes of coxae IV not fused to the apodemes near the genital slit; apodemes near the genital slit not contiguous with the apodemes of the suctorial plate. Some sclerotization present on coxal fields I and II and in an area extending from coxal apodemes III to the level of coxal fields IV. Suctorial plate small; median suckers much larger than the anterior ones; two pairs of conoidal setae situated, respectively, laterally and posteriorly to the median suckers; one posterior median and paired posterior lateral and anterior lateral cuticular suckers present. Genital papillae two-segmented, the apical segment tapering to a thin point. Setae *ga* elongate and filiform, inserted at the junction of the anterior coxal apodemes IV and the ventral apodeme; setae *g* short and filiform. Coxal setae *cx IV* minute, coxal setae *cx I* and *cx III* absent, represented by vestigial alveoli; setae *sh* small and filiform.

Legs (Fig. 5e-g). All segments of the legs free; anterior legs long and well developed, legs IV strongly reduced in size, the width of the tarsus exceeding the length. Chaetotaxy: trochanteral, femoral and genual setae as in the female. Setae *gT* and *hT* on tibiae I-II and seta *kT* on tibia IV short and filiform; seta *kT* on tibia III longer. Tarsi I-II with six setae: seta *d* long and whiplike; seta *e* very thin and short, its base contiguous with that of seta *d*; seta *f* foliate, distal in position, setae *ra* and *la* foliate but more basal; seta *wa* elongate and filiform. Tarsus III with seven setae: seta *d* long and whiplike; seta *f* foliate and distal; setae *e*, *w* and *r* foliate, on the basal 2/3 of the tarsus; seta *p* flattened, sometimes slightly foliate, apical; seta *s* spinose, almost reaching the tip of the tarsus. Tarsus IV with five setae: seta *d*, very long and whiplike, positioned dorsally on a pointed outgrowth of the tarsus; seta *w* also long and whiplike, but much shorter than *d*; seta *r* shaped like a blunt spine; seta *s* very small and filiform; seta *p* long and broad, distally attenuate (Plate Ie). Solenidia: solenidion sigma-1 on genu I distinctly shorter than sigma-1 on genu II; solenidion

sigma-2 on genu I very short (Plate Id). Tibial solenidion phi very long and tapering on tibiae I-II, shorter on tibia III and very small on tibia IV. Solenidion omega-1 on tarsus I as in female with a famulus epsilon; omega-2 vestigial, often absent and only represented by an unsclerotized spot (Plate Ic) posterior basal to omega-1; omega-3 long and tapering, positioned near omega-1. Solenidion omega-1 on tarsus II as on tarsus I, without famulus. Pretarsi I-III with an ambulacrum; empodial claw simple.

PROTONYMPH (Large type). Average length of 11 specimens 299, S.D. 13; average width 206, S.D. 11.

The protonymph differs from the female in the following characters: vulva and bursa copulatrix absent; only one pair of genital papillae; propodosomal shield absent; setae *ga* and *cx IV* absent; seta *d*₄ very small; trochanteral setae absent; chaetotaxy of leg IV reduced; femur and tibia bare, tarsus without seta *s*; solenidion omega-3 on tarsus I absent. Chelicera and gnathosoma as in female.

PROTONYMPH (Small type). Average length of four specimens 257, S.D. 12; average width for three specimens 156, S.D. 3.

This type of protonymph differs from the large type in its slightly small size (*P*-value < 0.10 (*d.f.*: 12) in Student's *t*-test) and longer dorsal setae (ratio of dorsal setae/body length 0.40–0.50, in large type 0.20–0.25; difference significant: *P*-value 0.003 (*d.f.*: (1, 4)) in three-way analysis of variance using the following setae: *sci*, *d*₁, *d*₂ and *d*₃). In all other aspects it resembles the large type protonymph.

LARVA (Large type). Average length of 11 specimens 192, S.D. 14; average width 126, S.D. 12. The larva differs from the protonymph in the following characters: legs IV absent; genital papillae absent; setae *d*₅, *l*₅, *a*₂ and *a*₃ absent; setae *d*₄ and *l*₄ elongate, filiform; solenidion omega-2 on tarsus I absent; seta *mG* on genua I-II spine-like. Claparede's organ absent.

LARVA (Small type). Only one, severely damaged specimen was available. It differed from the large type larva by its distinctly smaller chelicerae. No other differences were observed.

Host and locality

Allodynerus rossii (Lepelletier, 1841), THE NETHERLANDS, Zuid Holland prov., De Lier, 8-III-1983, coll. Brugman, host in collection of the Rijksmuseum van Natuurlijke Historie, Leiden (RNHL); same host species, THE NETHERLANDS, Noord Brabant prov., Hilvarenbeek, 8-II-1983, coll. De Ruijter, host in RNHL; AUSTRIA, Eichkogel, 8-VI-1949, coll. Kocourek, host in collection of the British Museum (Natural History), London (BMNH); BULGARIA, Sliven, 7-18-VII-1962, coll. O. Slaby, host in BMNH; GREECE, Thessalia, Kalampaka, 14-20-VII-1979, colls Day, Else & Morgan, two specimens, both in BMNH; TURKEY, Amasya prov., 22-24-V-1959, coll. Guichard, host in BMNH; FRANCE, Sèvres, host in collection of the Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts (MCZ).

Deposition of specimens

Voucher specimens of the mites are deposited in: Rijksmuseum van Natuurlijke Historie, Leiden; British Museum (Natural History), London; Museum of Zoology, University of Michigan, Ann Arbor, Michigan; U.S. National Museum of Natural History, Washington, D.C.; Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts; Cornell University Insect Collection, Ithaca, New York; Institut royale des Sciences naturelles, Brussels;

Field Museum of Natural History, Chicago, Illinois; Zoological Institute, Academy of Sciences, Leningrad; Acarology Laboratory, Ohio State University, Columbus, Ohio; Zoologisches Institut und Zoologisches Museum, Hamburg; Bernice P. Bishop Museum, Honolulu, Hawaii; Muséum Nationale d'Histoire Naturelle, Paris; and in the collections of the authors.

Discussion of morphology

No consistent differences were observed between the deutonymphs of the different populations collected during this study and the deutonymphs collected by Cooreman in Belgium (Klommen & O'Connor, In prep.). Comparison with the Zakhvatkin types was not possible since these types are lost. The mites examined in this study did differ from the description by Zakhvatkin in a few points: a fourth foliate seta is present on tarsus III of the deutonymph which was not mentioned in the original description. This error can be made readily, since foliate setae *e* and *r* of tarsi III tend to overlap on a microscope slide. It is more difficult to understand why Zakhvatkin omitted solenidion omega-2 from tarsus I, a solenidion which is clearly visible in all our specimens. It is especially intriguing since he did mention a spot indicating the position of solenidion omega-2 for *E. parasitica*, an observation that has not been repeated by any of the other authors describing this deutonymph (Vitzthum, 1925; Cooreman, 1942; Baker & Cunliffe, 1960), nor has it been observed in any of the specimens of this species collected during this study. Despite these differences, we are convinced of conspecificity of our specimens and those described by Zakhvatkin.

Life history

At the onset of our study period, early March, the wasp larvae were still inactive. A number of female and large type male mites moved around on these larvae. Several of the females carried an egg, which developed inside to a small type larva. This larva moulted to a small type protonymph, which was born. Without passing through a deutonymphal stage, the protonymph developed into a small type tritonymph, a stage observed, but not collected. The tritonymph produced a small type male. The slight increase in size from small type protonymph to male suggests that the small type nymphal stages are feeding.

The above part of the life cycle of the mites is not correlated to any discrete events in the life cycle of the wasp, but the development of the other stages does show such a correlation. In all cells observed, pupation of the wasp was followed within a day by the start of oviposition by the female mites. Each female produced about 20 eggs over a period of several days, deposited largely on the mouth parts and legs of the wasp pupa. The eggs hatched to free larvae of the large type which moulted to large type protonymphs by the time the pupa had darkened. Both stages stayed on the anterior part of the pupa. Upon eclosion of the wasps, the protonymphs were removed with the exuvial skin and moulted within one or two days to deutonymphs. A few protonymphs moulted while still on the pupa, but most of their exuvial skins were found on the shed skin of the pupa. Within two days after their moult, the deutonymphs gathered on the wasp, where they concentrated in the acarinarium at the base of the second abdominal tergum. Under natural conditions, the wasp may remain in the nest for three to four days before emerging (Crèvecoeur, 1945), but in this study both mites and wasps were killed a few days after eclosion.

The correlation in development of mites and wasps was maintained at the two different temperatures. Although the wasps developed substantially faster at 30 °C than at 20 °C, the

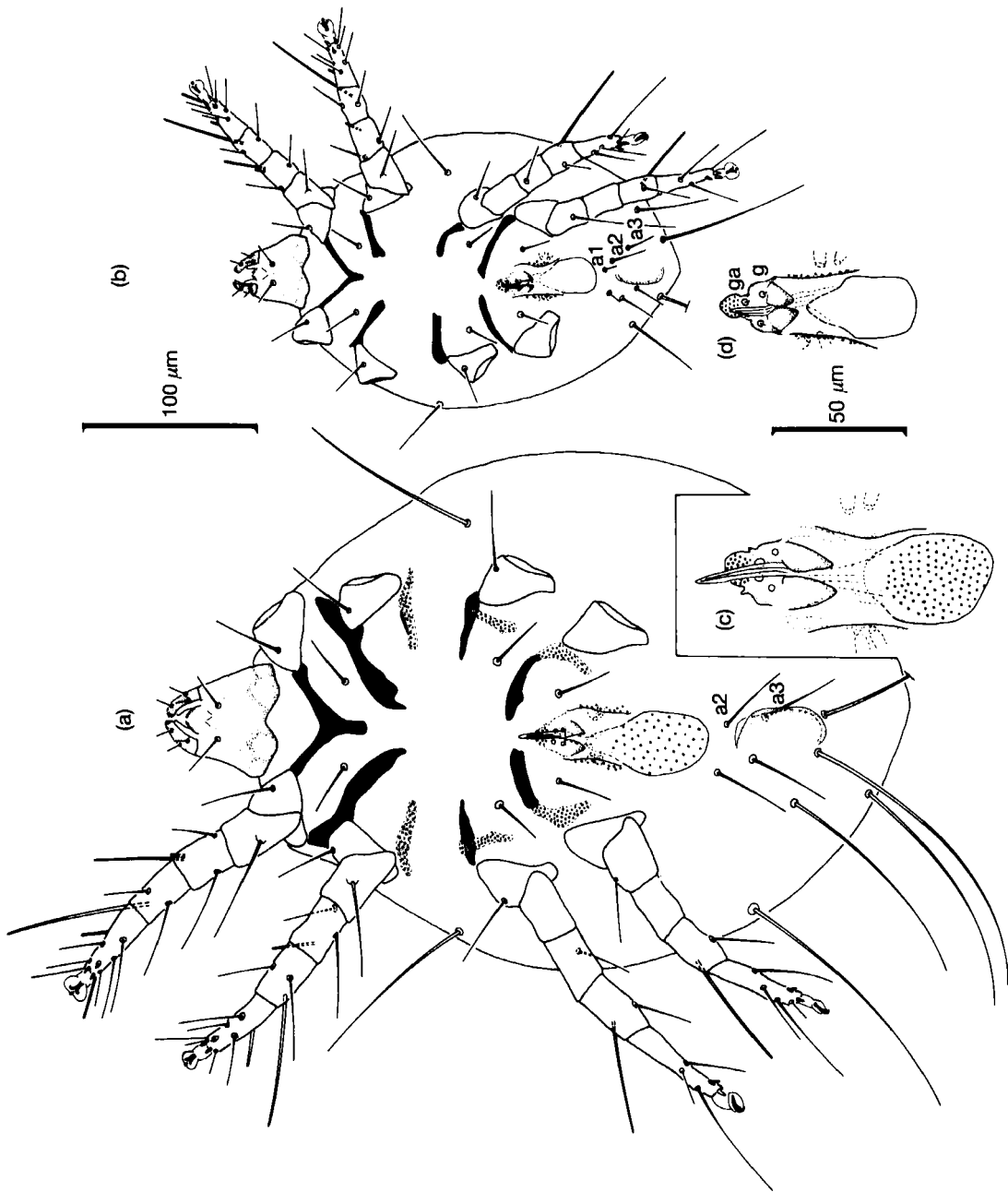


FIG. 3. (a-d) *Enstiniella kostylevi* Zakhvatkin, 1941. Male. (a) Large type male, ventral view; (b) small type male, ventral view; (c) large type male, genital region; (d) small type male, genital region.

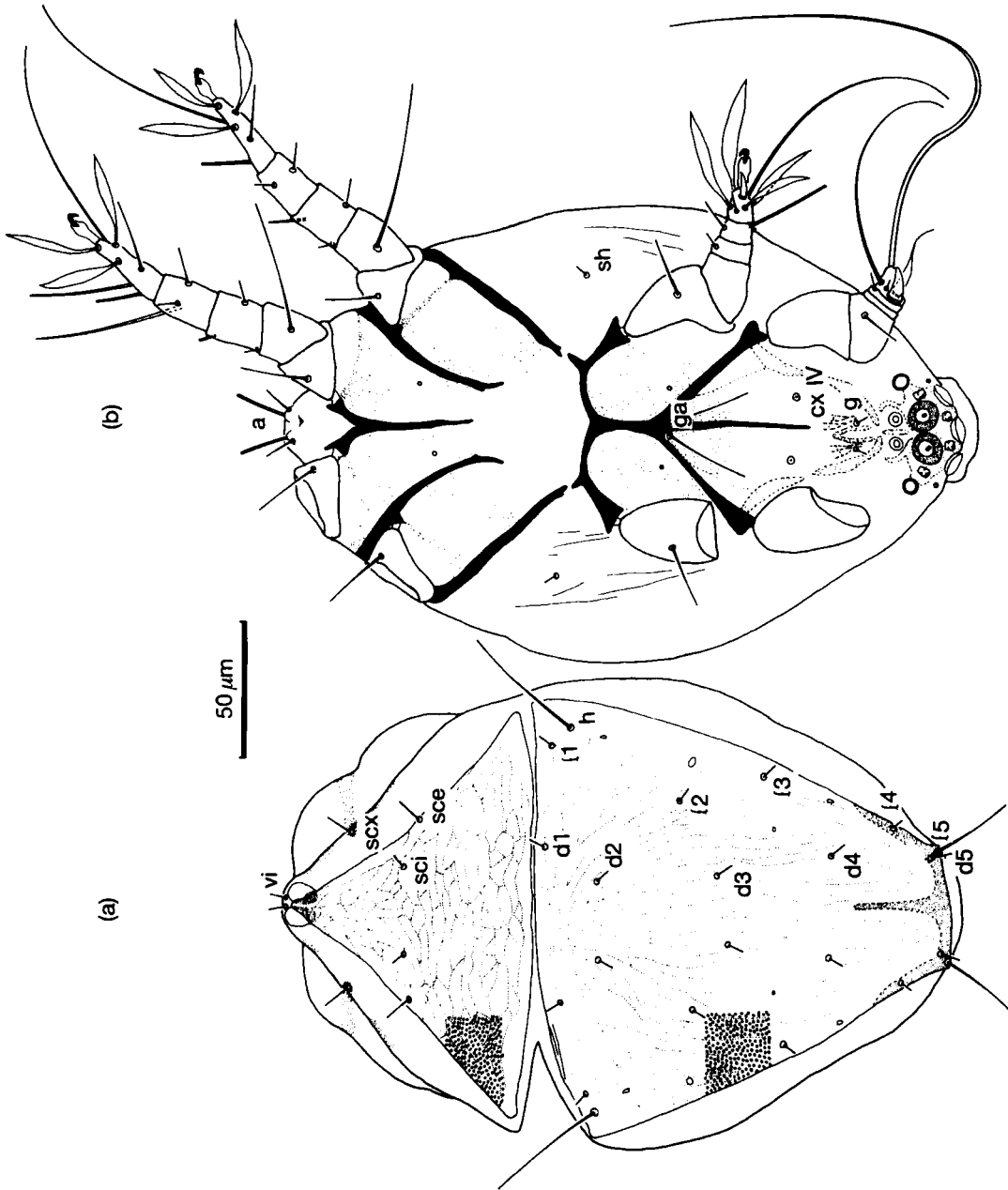


FIG. 4. (a, b) *Ensliniella kostylevi* Zakhvatkin, 1941. Deutonymph. (a) Dorsal view; (b) ventral view.

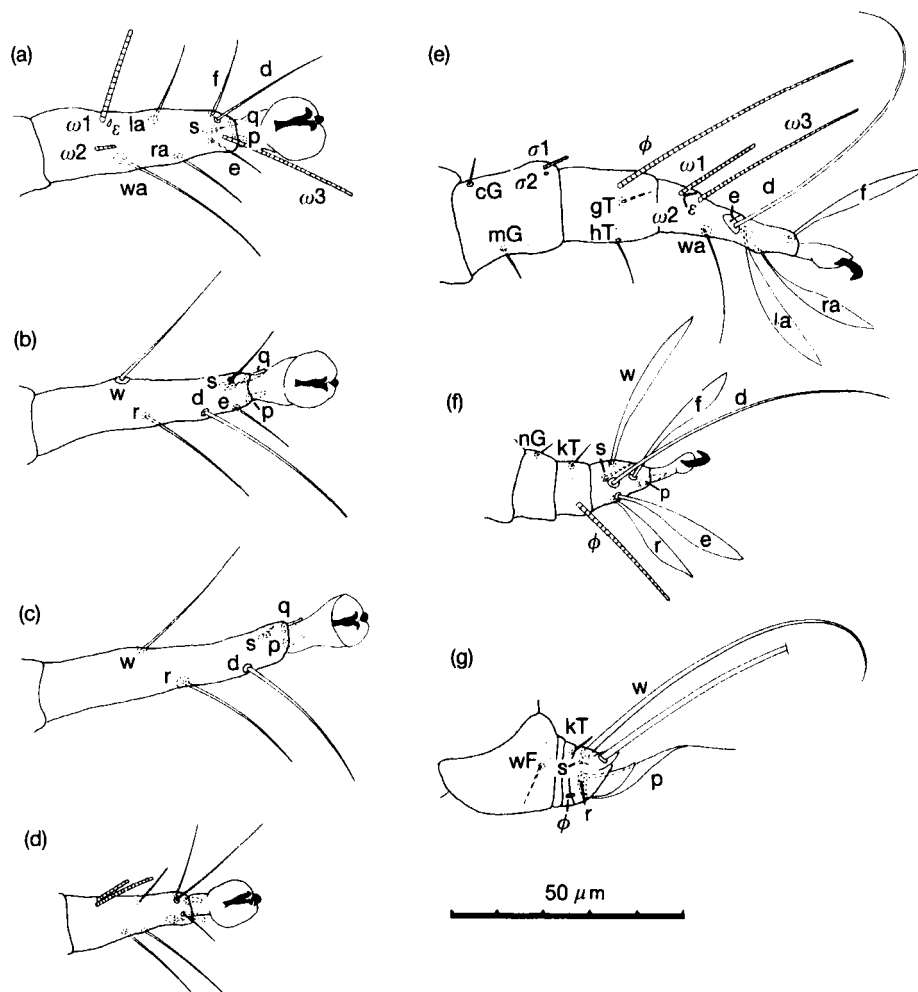


FIG. 5. (a-f) *Ensliniella kostylevi* Zakhvatkin, 1941. (a) Female, tarsus I dorsal view; (b) female, tarsus III; (c) female, tarsus IV; (d) small type male, tarsus II dorsal view; (e) deutonymph, leg I dorsal view; (f) deutonymph, leg III; (g) deutonymph, leg IV.

development of the mites was similarly accelerated. This agrees with the results of a similar experiment conducted on *E. parasitica* and its host *A. delphinalis* by Cooreman (1942).

A few observations on disturbances in normal development may further illustrate the importance of the host for the development of the mites. In one cell, the wasp larva was infected by a disease resembling foulbrood in honey bees. At the time it should have pupated, the larva produced a large quantity of exuvial fluids, but did not shed its larval skin. Within this skin, wasp development continued for a short period (coloration of the eyes, development of the head, etc.) after which the larva died. Meanwhile the female mites delayed oviposition (waiting for pupation?), but eventually produced a few eggs on the head of the larva. These hatched to normal large type larvae but did not develop any further and died soon after.

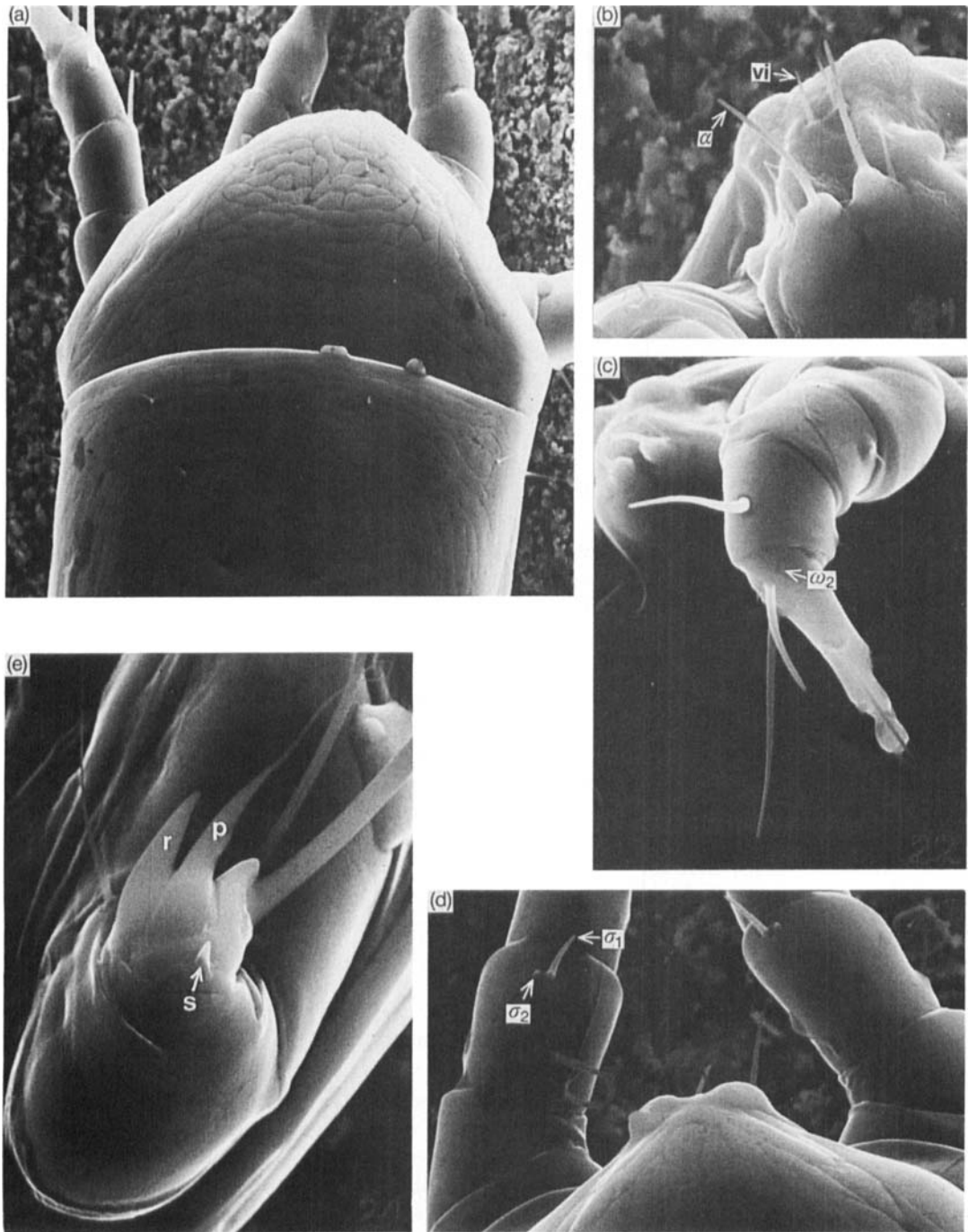


PLATE I. (a-e) *Ensliniella kostylevi* Zakhvatkin, 1941. Deutonymph. (a) Propodosoma, dorsal view; (b) gnathosoma; (c) tarsus and tibia I, dorsal view; (d) genu I, dorsal view; (e) tarsus IV, ventral view.

In a few other cells, the adult wasps were removed while the mite nymphs were still on the pupal skin. After three days all deutonymphs had left the pupal skin, running around in the dish in which they were kept. After four days the first deutonymphs became motionless, no longer responding to tactile stimuli. At day 8, after removal of the adult wasps, about half of the deutonymphs were dead. Although these observations in themselves do not allow any conclusions regarding the immediate cause of death of the mites, they both illustrate the importance of the hosts in all stages of development of the mites.

Discussion

The life history of *Ensliniella kostylevi* closely resembles that of *E. parasitica*, the only other species in the genus that has been studied with respect to its life history (Vitzthum, 1925; Cooreman, 1942). However, neither Vitzthum or Cooreman mention the existence of the small type stages, although this might be an artefact of the rather crude methodology employed. The small type stages do exist in *Vespacarus* Baker & Cunliffe, 1960 and *Kennethiella* Cooreman, 1954 (Krombein, 1967; Cowan, 1984), two genera that are closely related to *Ensliniella* (Klompfen & O'Connor, In prep.). Similarly, the development of these mites and their hosts is also closely correlated. Data on the life history of *Vespacarus* species are scant (Krombein, 1961, 1967), but seem to agree in nearly all aspects with those for *Ensliniella*. However, there are some notable differences in development and behaviour between *Kennethiella* and *Ensliniella*. In *Kennethiella*, the large type protonymphs often leave the host when they are fully engorged, clustering on the walls of the cell. Here they moult to deutonymphs, often a few days before eclosion of the wasp (Krombein, 1967; Cowan, 1984). The deutonymphs return to their host, but the correlation between the eclosion of the wasp and the moult to deutonymphs is less pronounced than in *Ensliniella* (present study) or *Vespacarus* (Krombein, 1961, 1967).

The observed correlation in development of the mites and their wasp hosts is hypothesized to be maintained by absorption by the mites of growth and differentiation hormones of the host (Krombein, 1961; Mostafa, 1970). This could happen during feeding, although the evidence is circumstantial: most stages of *Ensliniella*, *Vespacarus* and *Kennethiella* feed continuously on their hosts, with the stages that do not, like the large type protonymphs of *Kennethiella* after engorgement, showing distinctly less correlation in development. The feeding behaviour of the mites does not seem to have any deleterious effect on the development of the host (Krombein, 1961, 1967; Cowan, 1984; present study): no differences were observed between development of the wasps in cells with or without mites.

If the life histories of *Ensliniella*, *Vespacarus* and *Kennethiella* are compared to that of a more distantly related genus, *Monobiacarus* Baker & Cunliffe, 1960, a number of differences are apparent. In *Monobiacarus*, the small type stages are absent, and the development of mites and wasps is not correlated in any obvious manner (Krombein, 1967). Neither does *Monobiacarus* feed on its host in any stage; presumably they feed on organic debris in the cell (Krombein, 1967).

A major difference between *Ensliniella* and *Kennethiella* exists in the sex ratio of the oviparously produced adults, the females and large type males. Since the data available on this subject are largely restricted to *E. kostylevi* and *Kennethiella trisetosa* Cooreman, 1942, the following discussion will focus on these two species. Both females and large type males develop from the deutonymphs invading the cell during oviposition of the wasp. In *K. trisetosa*, the percentage of deutonymphs developing into males is about 9% (Cowan, 1984); in *E. kostylevi* this figure is about

44% (51 large type males and 65 females were recovered). In order to propose an hypothesis explaining this difference, a few other factors have to be considered.

The number of deutonymphs (founders) of *K. trisetosa* invading each cell is very low, on average 2.3 mites per cell with a range of 1–8 in a total of over 100 cells examined (Krombein, 1967; Cowan, 1984). The number of founders in *E. kostylevi*, *E. parasitica* (Vitzthum, 1925; Cooreman, 1942; Benno, 1945; Crèvecoeur, 1945) or *Vespacarus* species (Krombein, 1967) is much higher, ranging from 5–15 per cell. This difference appears to be related to the mode of transportation of the deutonymphs on the female wasp. The nymphs of *K. trisetosa* are packed in the genital chamber of the wasp, and the female wasp kills most mites on its exterior (Cooper, 1955). The number of mites able to invade a single cell is therefore limited, since they can only leave the genital chamber during oviposition of the wasp. The deutonymphs of *Ensliniella* and *Vespacarus* are transported in acarinarium on the abdomen of the wasp, which can be closed if the wasp stretches its abdomen straight backward, but which will still offer the mites a much better chance to invade a cell in larger numbers. Furthermore, no antagonistic behaviour of the female hosts has been reported against these mites.

Another issue relevant to the sex ratio question is the function of the small type male. We agree with Cowan (1984) that the small type male probably evolved to ensure that every female would mate and reproduce, even if she ended up in a cell without (large type) males. If, as observed in *K. trisetosa*, the small type males mate randomly with the females available in a cell, they can also ensure outbreeding (Cowan, 1984). The only remaining reason for the large type males to persist in the population appears to be temporal: in *K. trisetosa* they mate before the small type males are fully developed and will thus always outcompete the small type males if present in a cell (Cowan, 1984). Although this behaviour was not observed in *E. kostylevi*, we assume a similar situation.

Given the above observations, the difference in the sex ratio of the oviparously produced adults of *E. kostylevi* and *K. trisetosa* can be explained in terms of the number of founders, and, indirectly, as a result of morphological and behavioural characteristics of their wasp hosts. The low number of founders in *K. trisetosa* strongly increases the probability of having cells with mites of only one sex. This implies that the advantage of producing large type males is small, since: (1) large type males ending up alone or with other males in a cell will not reproduce; and (2) many females will not mate with a large type male because there are none in their cells. Under these conditions, the individual fitness of a female producing offspring with a bias towards females will be higher than that of one producing offspring with an unbiased sex ratio.

In *E. kostylevi*, the probability of ending up in a cell with mites of only one sex is small, due to the considerably higher number of founders. The observed subequal ratio of large type males to females can be explained by a mechanism analogous to Fisher's arguments for equal sex ratios in populations with panmixia (Fisher, 1958). In this species, a female will attain highest fitness if her offspring have an unbiased sex ratio. Data on the sex ratios in *Vespacarus* species are not available, but we predict subequal ratios of large type males to females based on the high number of founders.

A major assumption implicit in the proposed hypothesis is that a substantial degree of outbreeding exists. High levels of inbreeding are usually associated with a decrease in the number of males, caused by avoidance of sibling competition (Hamilton, 1967). In the mite-wasp system, high levels of inbreeding would be expected to result in the virtual disappearance of the large type male. However, this stage is quite numerous in *E. kostylevi*. Secondly, the observation of frequent fighting among the small type males of *K. trisetosa* (Cowan, 1984) is indicative of existing outbreeding in this species (Hamilton, 1967). The large type males of *K. trisetosa* do not fight,

presumably as a result of the very low frequency at which two or more large type males will be present in a single cell (Cowan, 1984). Outbreeding could occur in two ways: in the nest, if more than one female wasp deposited eggs in the same nest, or on the wasp, by exchanges of deutonymphs from wasp to wasp. The first mode has never been demonstrated, but it has been observed that *Allodynerus delphinalis*, the host of *E. parasitica*, frequently takes over nests of other Hymenoptera (*Osmia*, *Trypoxylon*) (Enslin, 1922; Benno, 1945; Crèvecoeur, 1945). Displacement of females of the same species has not yet been reported. Exchange of mites from wasp to wasp has been observed for *K. trisetosa* during mating of the wasps (Cooper, 1955; Cowan, 1984). Krombein (1967) suggested a similar mechanism for *Monobiacarus*, but he rejected it for *Vespacarus*. Studies on the behaviour of *Ensliniella* deutonymphs on the wasps are at present unavailable.

It does not seem appropriate to try to explain this system in terms of the existing models for the evolution of biased sex ratios. In fact, there is no biased sex ratio if the number of all functional males (large type + small type) is compared to that of females. There are a number of other problems in applying these models to this mite-wasp system. The group selection model (Wilson & Colwell, 1981; Colwell, 1981) assumes that the individual fitness of a female producing offspring with an unbiased sex ratio will always be higher than that of a female producing offspring with a biased ratio. As argued above, this is not correct in this system for situations in which the number of founders is low. This makes group selection, if present, indistinguishable from individual selection.

The Local Mate Competition model (Hamilton, 1967) is based on the idea that biased sex ratios will evolve under conditions of high levels of inbreeding to avoid competition between sibling males. Such high levels of inbreeding have not been established in this mite-wasp system and, secondly, if the biased ratio in *K. trisetosa* is a result of avoidance of competition between large type males and, either other large type males (brothers), or small type males (nephews), then it is not evident why the ratio in *E. kostylevi* is not biased to the degree observed in *K. trisetosa*. Until more data on the different aspects of the mite-wasp system become available, it seems more parsimonious to explain the system in terms of numbers of founders.

Insofar as the evolution of the mite-wasp system is concerned, one may assume that the male dimorphism arose in a mite species with low numbers of founders. Under such conditions, selection would favour the evolution of the small type male (or of complete theletoky). The genera *Ensliniella*, *Vespacarus* and *Kennethiella* form a natural group (Klompén & O'Connor, In prep.) and the life history as observed in *K. trisetosa* is probably close to that of their common ancestor in which the male dimorphism arose. In systems like those of *Ensliniella* or *Vespacarus* in which the number of founders is high, the presence of the small type males is no longer essential and the loss of the capability to produce these males might be expected. It is therefore not surprising that some females of *E. kostylevi* do not seem to produce a small type male any more, while all females of *K. trisetosa* do produce one, independent of the presence of large type males (Cowan, 1984). Although no counts were made in the cells of *E. kostylevi*, the number of small type protonymphs and males recovered (four of each compared to 65 females) strongly suggests that some females do not produce small type males. This suggestion remains valid even if we assume that a number of females were recovered before the development of even a small type larva, and if we allow for a high mortality rate among the small type males (Cowan (1984) found high mortality rates among small type males of *K. trisetosa* due to frequent collisions). An additional test for this hypothesis can be made involving *Vespacarus* species, by determining whether or not all female *Vespacarus* produce a small type male.

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